

Morphological survival of twice-cryopreserved human embryos.

Lierman S., De Croo I., Heindryckx B., Van den Abbeel E. and De Sutter P.

Centre for Reproductive Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

Introduction:

The role of frozen-thawed embryo transfer (FET) has increased in assisted reproductive technology programs because ET policy currently favours single ET. In FET, when cryopreserved oocytes, embryos or blastocysts are thawed, there may be surplus thawed/warmed oocytes, embryos or blastocysts available after transfer. These may be refrozen at the blastocyst stage for future clinical use. In this way the re-freezing procedure would be a valuable option to increase the cumulative pregnancy rate while decreasing the risk of multiple gestations. The aim of the present study was to evaluate the efficiency of vitrifying blastocysts derived from frozen-thawed cleavage stage embryos.

Materials and Methods: Patients donated embryos for research which were left-over after fresh transfer and of insufficient quality to be frozen (= control embryos) or which were frozen-thawed and of insufficient quality to be transferred (= study group). All used research embryos were obtained after informed consent of the patients (Approved by the Federal Ethical Committee Adv027 and the local Ethical Committee 2009/660). Control and study group cleavage-stage embryos were further cultured in vitro and vitrified at the expanded blastocyst stage. All expanded blastocysts were vitrified after artificial shrinkage (AS) of the blastocoelic cavity by means of mechanical pipetting. Collapsed blastocysts were equilibrated and vitrified in an ethylene glycol – dimethylsulphoxide – sucrose based solution (*Mukaida et al. 2006*) and by using a closed CBS HS device (Cryo Biosystem). Vitrified collapsed blastocysts were stored in liquid nitrogen. After warming, blastocysts were transferred to solutions of different concentrations of sucrose to dilute the cryoprotectants (*Mukaida et al. 2006*). After dilution they were further cultured in vitro.

The main outcome measures were the morphological survival rates of the blastocysts after 2-3hrs and 24hrs. Morphological survival was defined as the percentage of fully intact and moderately damaged blastocysts. After 24hrs we also looked at the expansion/re-expansion status of the blastocoelic cavity.

Results: During the study period 162 blastocysts derived from fresh embryos (= control group) and 90 blastocysts from frozen-thawed cleavage stage embryos (= study group) were used. In both groups and before vitrification, the quality of the trophectoderm and the inner cell mass cells in the blastocysts was evaluated according to the scoring system described by Stephenson et al. (2007). Blastocysts with minimal grade C trophectoderm and grade C inner cell mass were vitrified. Blastocysts with minimal grade B trophectoderm and grade B inner cell mass were considered as good quality blastocysts. The percentage of blastocysts with morphological survival after 2-3hrs and 24hrs was not different between the study group and the control group (71.1% (64/90) vs. 78.4% (127/162), $p = 0.22$ and 66.7% (60/90) vs. 72.8% (118/162), $p = 0.32$). Furthermore, the percentage of good-quality blastocysts with morphological survival after 24hrs was not different between the study group and the control group (68.4% (52/76) vs. 76.3% (74/97), $p = 0.30$). After 24hrs of in vitro culture, the percentage of fully expanded, hatching or hatched blastocysts obtained after 24 hrs of culture was not different between the study group and the control group (46.7% (28/60) vs. 42.4% (50/118) for fully expanded blastocysts, 30.0% (18/60) vs. 26.3% (31/118) for hatching blastocysts and 13.3% (8/60) vs. 16.9% (20/118) for hatched blastocysts.

Conclusion: Our results show that blastocysts derived from frozen-thawed cleaved stage embryos can successfully be cryopreserved a second time by vitrification. The re-

cryopreservation by vitrification could be a valuable option to increase the cumulative pregnancy rate while decreasing the risk of multiple gestations, but still needs to be approached with some caution because little data is available on the long term safety of multiple freezing.